

# STUDIES ON THE EFFECTS OF PROGESTERONE ON THE PHYSIOLOGY OF REPRODUCTION IN THE MINK, *MUSTELA VISON*

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Reproduction in mink has been a major problem of the fur farmer since he began raising mink in pens. The two factors which contribute to the difficulty of getting good production are (1) a large number of "misses" or whelping failures and (2) litters of small size.

The mink exhibits a delayed implantation which, according to Hansson (1947), leads to a discontinuous embryonic development and a great variation in length of the gestation period. The length of delay in implantation varies widely and seems to be correlated with time of mating. Hansson showed that in mink there is a definite relationship between the date of mating and the length of gestation in that animals that had been bred early had longer gestation periods. This delay in implantation had been observed in other animals much earlier. Bishchoff (1854) reported on delayed implantation in roe deer, *Capreolus*. Fries (1880) recorded a delayed implantation in the European badger, *Meles*. Lataste (1891) discussed five species of rodents in which delayed implantation occurred. Patterson (1913) described the early development of the polyembryonic armadillo, *Dasypus novemcinctus*, and announced a quiescent uterine period immediately preceding implantation.

It appears that this delay could result in a number of conditions which would be disadvantageous to a successful breeding season. The delay may result in death or resorption of blastocysts which could result in a whelping failure or a reduction in litter size.

According to Hansson, if a mated polyestrous animal again came into estrus, this was due either to the egg not having been fertilized or to conditions not being favorable for implantation after fertilization. If implantation does not occur, the characteristic changes accompanying it fail to appear. The fact that a polyestrous animal comes into estrus after mating need not indicate that the eggs from the preceding mating were either not fertilized or were not in a condition for implantation, but may be due to the uterine lining not having been in optimum condition for implantation. Ball (1940) showed that this is often the case with rats, in which copulation does not always elicit corpus luteum activity, with the result that the eggs cannot be implanted although they are fertilized and have developed up to the implantation stage.

Several workers have considered that the delayed implantation is due to a disturbance in the internal secretions, but opinions are very divergent on this point. Some believe that it is caused by too low a production of gonadotropic hormones from the anterior pituitary; some feel that the estrogen production is too low; and still others believe that the corpora lutea do not produce sufficient progesterone.

Since a great deal of work had been done in attempting to overcome the delay in implantation without success, it occurred to this writer that some of the associated phenomena might be changed. It was first suggested by Hammond (1951) that the injection of progesterone after mating might result in an increase in litter size. Since a difference in mean litter size has been observed among different color mutations, it was decided to test the possibility of a genetic deficiency of activity of the corpora lutea linked to the various color mutations. Another

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facet of this problem could be the reduction of the number of "misses" by these injections. In conjunction with these problems would be the study of the histological effects of these injections on the corpora lutea.

#### MATERIALS AND METHODS

The mink used in this experiment were donated to The Ohio State University by members of the Ohio Mink Breeders' Association. The experiment was carried out over two breeding seasons in order to accumulate sufficient data to be treated statistically.

During the first year, animals were treated on four different ranches. The animals treated on two of the ranches consisted of a variety of color phases. These two populations were made up of sapphire, aleutian, pastel, dark, blue frost, white, and silverblu mink and contained kit females as well as older females. One of the other two groups consisted of kit sapphire females, whereas the remaining group contained only kit pastel females. In the latter two groups a single color phase was used in order to determine if a genetic deficiency of progesterone production might be linked with a particular color mutation. In all four experimental groups there were 47 treated animals and 51 control animals. Wherever possible, sisters of the experimental animals were used as controls. The experimental animals were given a single injection of 5 mg of progesterone in oil. The injection was made intramuscularly in the thigh 6 to 8 days after copulation. The time of injection was based on Enders' (1952) and Hansson's work in which they stated that the time elapsed during passage of ova through the tubes was 6 to 7 days.

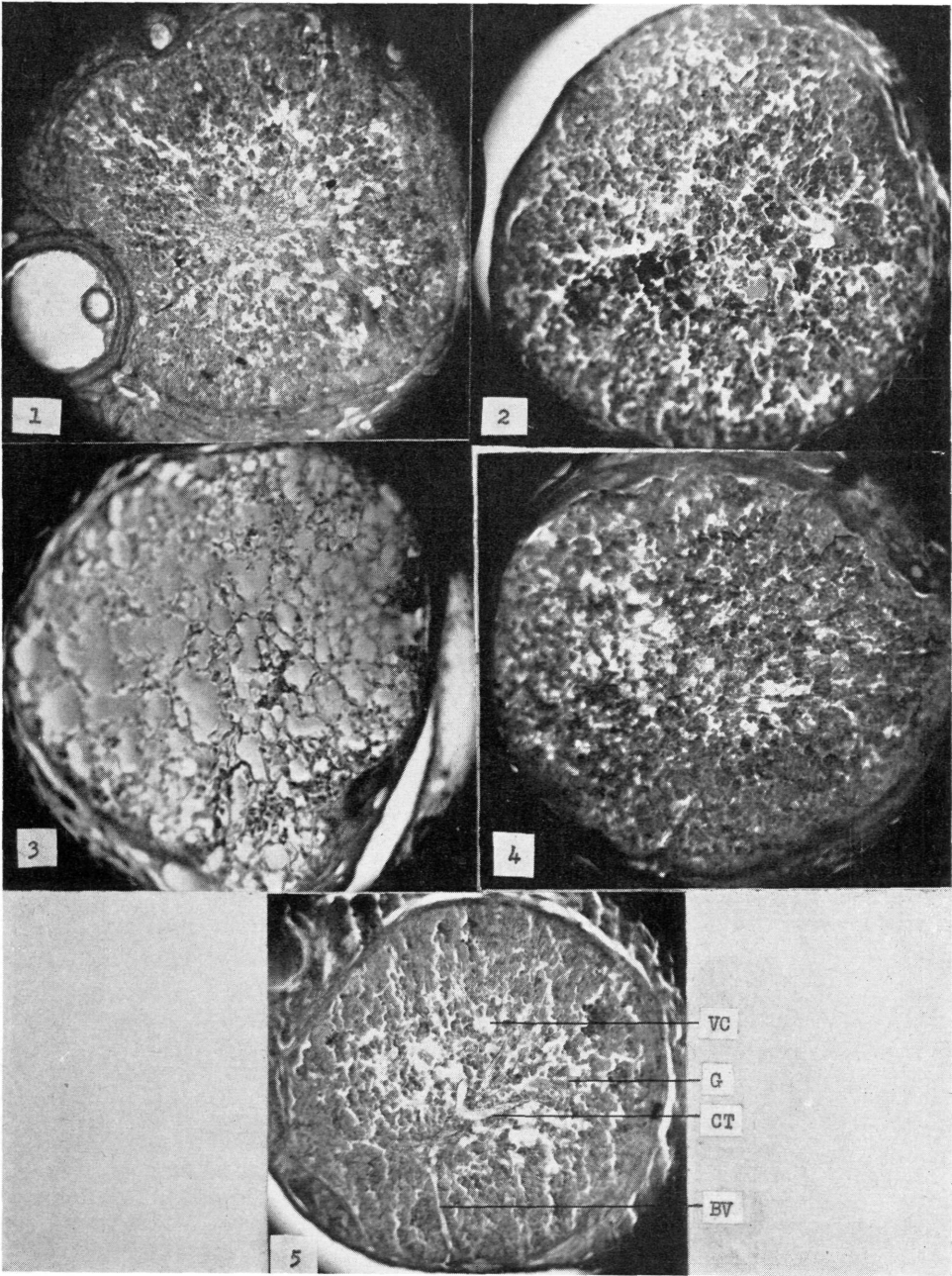
The following year arrangements were made with various mink ranchers throughout northern Ohio and two ranches in Illinois whereby the writer would furnish instructions and progesterone in return for accurate whelping records. During this breeding season the work was continued at the local mink ranch. The author made all injections on the latter ranch. From the accumulated data, a statistical analysis of the effect of injection on the number of whelping failures was made. In this group there were 1,234 females, 727 being injected with 5 mg of progesterone in oil and 507 used as control animals and not injected. Again, sisters of injected animals were used as controls whenever possible.

In attempting to discover possible effects on the histology of the corpus luteum, a number of animals were sacrificed at various times after breeding and the ovaries were recovered. The animals used in this phase of the experiment also furnished material for another investigator who was making a study of the embryology of the mink. For this reason, the animals were opened and one uterine horn was removed. At a later time these same animals were reopened and the second uterine horn and both ovaries were removed. The operative technique consisted of anaesthetizing the animal by the intraperitoneal injection of 0.35 cm<sup>3</sup> Nembutal Sodium solution, 60 mg/cm<sup>3</sup>. Surgical hair clippers were

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#### EXPLANATION OF FIGURES IN PLATE

1. Photomicrograph of a corpus luteum of an injected animal. Actual maximum diameter was 1.9 mm. Note denser peripheral arrangement of granulosa cells and section of a blood vessel in lower right area. At lower left is an ovum in unruptured follicle.
2. Photomicrograph of a corpus luteum of a control animal. Actual maximum diameter was 1.9 mm. Note uniform density of granulosa cells interspersed with few vacuoles.
3. Photomicrograph of a corpus luteum of an injected animal. Actual maximum diameter was 2.2 mm. Note the extreme vacuolation of the entire corpus luteum.
4. Photomicrograph of a corpus luteum of a control animal. Actual maximum diameter was 2.2 mm. Note lack of extensive vacuolation and distribution of vacuoles present.
5. Photomicrograph of a typical corpus luteum selected to show granulosa cells, blood vessels, and general picture of a functional corpus luteum.



then used to remove the fur from the ventral pelvic region. A 1 and  $\frac{3}{4}$  inch incision, performed to the right of the linea alba, provided sufficient exposure of the organs involved. Since the first incision was made to obtain one of the uterine horns for the embryological study, the ovary and oviduct were left intact after the excision of the horn. This excision was accomplished after ligating the major blood vessels supplying and draining the uterine portion to be excised. In the few instances of excessive bleeding the application of a Thrombozyme-soaked cotton swab effected coagulation.

It was found that during the course of the operations strict aseptic practices were unnecessary and were confined to bathing instruments and hands in 85 percent ethyl alcohol. The incised abdominal wall was sutured with surgical thread and the cut edges of the skin were drawn together with metal wound clips. Shackelford (1952) noted difficulty in maintaining surgical closure with thread or catgut due to the animal's tendency to gnaw or claw at the incision area. No difficulty was experienced from this type of behavior after using the metal clips. At a later time, the interval of which was determined by the embryologist, the animal was sacrificed by giving an overdose of nembutal and the remaining uterine horn and the ovaries were quickly removed and placed in Bouin's fixative. The ovaries were removed from the fixative after 48 hours of fixation and stored in 70 percent alcohol. The ovaries were then treated with lithium carbonate solution to facilitate removal of the traces of the fixative (picric acid crystals) and then imbedded in paraffin after treatment in the regular alcohol series. They were then sectioned at 50  $\mu$ , and whenever possible, these sections were mounted in serial order. The tissue was stained with Ehrlich's glycerine-alum haematoxylin and counterstained with eosin after which they were mounted in piccolite. The slides were studied microscopically and all corpora lutea were traced from their first appearance in the sectioned ovary to their disappearance from later sections. The corpora lutea were measured at their greatest diameter by using a vernier caliper under 30X magnification. Measurements were made to the nearest one-tenth of a millimeter. The practice of following each corpus luteum through the ovary via the serial sections made it possible to count the total number of corpora lutea. The number of corpora lutea per ovary varied from 0 to 20. Their size varied from 1.4 mm to 2.6 mm. Ten slides were selected at random from those comprising the treated group and ten slides from those of the control group. Photomicrographs were made of the various corpora lutea on these slides in order to compare histologically the corpora lutea in these two groups. Those corpora lutea that were photographed were selected as the largest represented on that particular slide. Plate I shows typical sections of corpora lutea from treated animals as compared to those from control animals.

#### RESULTS AND DISCUSSION

*Effects of treatment on litter size.*—During the first year the animals were treated on four ranches with the animals designated as Groups 1, 2, 3, and 4. In Group 1 (U-55) were 8 treated and 7 control animals. The treated animals averaged 4.75 kits per litter; the control animals averaged 4.29 kits per litter. In Group 2 (N-55) 9 treated animals averaged 4.33 kits per litter; 9 control animals averaged 3.0 kits per litter. In Group 3 (G-55) 15 treated sapphire females averaged 4.6 kits per litter; 15 untreated kit sapphire females averaged 3.53 kits per litter. In Group 4 (S-55) 15 treated kit pastel females averaged 5.63 kits per litter; 20 untreated kit pastel females averaged 3.90 kits per litter. These latter two groups were used to test the contention that a genetic link between low corpus luteum activity and color mutation existed. The fact that the pastel animals responded more markedly than the sapphire animals seems to support the assumption that such a genetic link exists.

The following breeding season on the neighborhood ranch (N-56), a mixed

group of fifty-seven treated and thirty-three control animals were used. The discrepancy in the size of the two groups was due to the manner in which litter-mates were used. Where three females occurred in a litter, two were used as treated animals and the other as a control. The treated animals produced 227 kits, an average of 3.98 kits per litter. The control group produced 67 kits, an average of 2.03 kits per litter.

These data were treated statistically using an analysis of variance. Table 1 shows the results of this analysis for the five groups described above. The F value derived in the first four of these groups was not statistically significant but

TABLE 1

*Individual treatment of the five groups showing the derived F value*

Group	Treated			Control			F
	N	$\Sigma x$	$\Sigma x^2$	N	$\Sigma x$	$\Sigma x^2$	
U-55	8	38	256	7	30	142	0.12
N-55	9	39	181	9	27	123	2.37
G-55	15	69	407	15	53	277	1.33
S-55	15	83	523	20	78	434	3.90
N-56	57	227	1155	33	67	259	18.74

TABLE 2

*Treatment of four groups as a single group*

Treated			Control			Total		
N	$\Sigma x$	$\Sigma x^2$	N	$\Sigma x$	$\Sigma x^2$	N	$\Sigma x$	$\Sigma x^2$
47	229	1367	51	188	976	98	417	2343

Source	S.S.	df	M.S.	F	F <sub>.05</sub>	F <sub>.01</sub>
Total	568.62	97				
Between	34.41	1	34.41	6.18	3.94	6.91
Within	534.21	96	5.565			

in every case there was a definite increase in litter size. The fact that these groups were made up of small sample sizes may account for an F value that was not significant but since there was an increase in litter size each time, the author felt that a statistical test of the combined results should be performed. Table 2 shows the results of the analysis of the first four groups. The F value derived was 6.18. The significant F value for this analysis using the appropriate degrees of freedom is 3.94 at the 5 percent confidence level and 6.91 at the 1 percent confidence level. The nearness of the determined value and the table value prompted still another combination of results. Table 3 shows the treatment of all five groups which yields an F value of 15.99 and the corresponding table value using the appropriate degrees of freedom is 6.77 at the 1 percent level of confidence.

The increase in litter size might be accounted for as being due to a decrease in loss of blastocysts through facilitation of implantation or a decrease in number of blastocysts resorbed. Enders reports that approximately 50 percent of the eggs ovulated fail to implant and are resorbed.

*Effects of treatment on number of whelping failures.*—That portion of the experiment dealing with the effect of the injection on the number of "misses" was performed during the 1956 breeding season. A total of 1,234 animals was used in this phase. These animals were separated into two groups, the treated group contained 727 and the control group 507. One hundred "misses" occurred in the treated group, 97 occurred in the control group.

The effects of the injection on the number of whelping failures or "misses" supports the idea advanced above concerning the mode of action of the hormone. Since it has been supposed that the injection renders the endometrium more nearly in optimum condition, thereby allowing more complete implantation, it is considered a reasonable conclusion that this is the explanation for the decrease in the number of "misses" in the treated group as compared to that in the control group. If the implantation is more complete, it is logical to assume that there

TABLE 3  
*Treatment of five groups as a single group*

Treated			Control			Total		
N	$\Sigma x$	$\Sigma x^2$	N	$\Sigma x$	$\Sigma x^2$	N	$\Sigma x$	$\Sigma x^2$
104	456	2522	84	225	1235	188	711	3757

Source	S.S.	df	M.S.		
Total	1068.06	187		F	F <sub>.01</sub>
Between	84.55	1	84.55	15.99	6.77
Within	983.51	186	5.287		

is a decrease in possible resorption or abortive blastocysts. The fact that progesterone is necessary for progestational proliferation (Corner and Allen, 1929; Block, 1939) supports this idea. It must be born in mind that progesterone also aids in decreasing uterine motility (Allen and Reynolds, 1935) thereby facilitating implantation and resulting in more complete or "solid" implantation.

The number of "misses" in the treated group was compared to the number of "misses" in the control group and treated statistically by using the chi-square test. This treatment yielded a chi-square of 6.44. This causes us to reject the null hypothesis which states that there was no difference in the proportion of "misses" in the treated group and in the control group. This further means that the value of chi-square obtained in this 2 x 2 table treatment is significant at the two percent level of confidence. Therefore, we would expect a value of chi-square of this magnitude to occur through chance in only two percent of the cases if the null hypothesis were true. Table 4 shows statistical treatment of this phase of the experiment.

*Histological effects of treatment.*—For this histological study, a survey was made of the slides recording the various conditions observed in each slide. This was

done with complete disregard of whether the slide was from a treated animal or control animal.

These slides were then sorted into two groups: the slides from the treated animals in one group and the slides from control animals in the other. From these slides, ten were selected at random from each group. The corpora lutea were measured on each and a photomicrograph of the largest corpus luteum on each of these slides was made. These slides were then studied again and comparisons were made with reference to the size of the corpora lutea, the degree of vacuolation, the condition of the blood vessels supplying and draining the corpora, and the general condition of the granulosa cells. Typical photomicrographs are shown in figures 1 through 4; figure 5 shows characteristics of corpora lutea mentioned above.

TABLE 4  
*Effect of treatment on number of "misses"—1956*

	Whelped	Misses		
Treated	627 A	B	100	T <sub>3</sub> 727
Control	410 C	D	97	T <sub>4</sub> 507
	T <sub>1</sub> 1037	T <sub>2</sub> 197	Total=N 1234	

$$\chi^2 = \frac{(AD-BC)^2 N}{T_1 T_2 T_3 T_4}$$

$$\chi^2 = 6.44 \text{ (Significant at 2\% level)}$$

$$df = 1$$

The comparative histological study demonstrated that in the treated animals the corpora lutea show a greater degree of vacuolation than in the control animals. The granulosa cells were found to be less compact in the interior of the corpus luteum in the treated animals than in the control animals. This condition was also accompanied by a greater degree of vascularization in the corpora lutea of the treated animals. This leads us to infer that the mode of action of the injected hormone is upon either the corpus luteum directly or through the pituitary causing an increase in the production of luteotrophin. This in turn stimulated the granulosa cells resulting in an increase in lipid deposition in these cells which is associated with the incretion phase. The presence of the lipid material in the granulosa cells is correlated, according to Hansson, with the "incretion" or active phase. He described five phases of luteal condition and his description of the "incretion phase" is comparable to that noted in the treated animals. That in the control animals compared to his description of the "resting phase." The granulosa cells of the injected animals were larger and more dense peripherally than those of the control animals. The granulosa cells of the control animals were uniformly distributed throughout the corpus luteum. There was a tendency for the corpora of injected animals to show a greater degree of vacuolation than those of control animals. This was observed through reading the slides in such a way as to be oblivious to whether the slide being read was from a treated animal or a control animal. Even though capillaries were usually visible infiltrating the

corpora of both the treated and control animals, there seemed to be a greater overall development and establishment of these capillaries in the corpora of the treated animals. The observation or visibility of the capillaries became increasingly more difficult as the degree of vacuolation advanced beyond a general distribution among the granulosa cells. In a number of slides, the corpora of the control animals were found to be infiltrated with spindle-shaped connective tissue cells. This infiltration is accompanied by the capillary network formation. The most striking difference between corpora of injected animals and those of control animals was the fact that the granulosa cells were less dense in the central portion of the corpus of the former. This seems to be correlated with the onset of activity of the central granulosa cells resulting in their increase in size thereby causing that area to have less nuclear material in relation to the amount of cytoplasm. This allows more light to pass through this area giving the impression of less density.

#### SUMMARY

Bred mink were given a single intramuscular injection of 5 mg progesterone in oil 6 to 8 days after the second breeding. This injection of progesterone causes an increase in litter size. This increase is more pronounced in some color mutations than others. The progesterone caused a decrease in the number of "misses" or whelping failures and caused histological changes in the corpus luteum.

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